the extracellular region. Rewiring effect of inter-residue interactions in 493R mutant pocket allosterically propagates across the channel resulting in a more stabilized global conformational ensemble of the channel. These findings predict a novel mechanism of ENaC's constitutive activity, in which changes in local dynamics can affect the relative population of the channel's active states and its open probability.

**2949-Pos Board B379**

**Role of Threonine 338 in CFTR Gating**

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As a member of the ATP-binding cassette (ABC) protein superfamily, CFTR, a phosphorylation-activated but ATP-gated chloride channel, is comprised of two transmembrane domains (TMDs) that form a gated anion conducting pore and two nucleotide binding domains (NBDs) whose dimerization/dissociation is allosterically coupled to opening/closing of the gate. Recent cysteine scanning of CFTR's TMDs not only identified pore-lining residues, but also suggested molecular motions of the TMDs involved in opening/closing of the ''gate''. Since many of the pore-lining residues exhibit clear state-dependent exposure to the aqueous pore, it is predicted that mutations at these positions might affect gating by altering the free energy level of a particular state. T338 was chosen because this pore-lining residue is mostly concealed from the pore in the closed state. We converted T338 to various amino acids and found that the physical properties of the side-chain at this position indeed affect CFTR gating as well as anion conductance. For hydrophilic residues like threonine itself ($0.57 \pm 0.06$, $n = 4$), serine ($0.45 \pm 0.02$, $n = 12$) and asparagine ($0.88 \pm 0.02$, $n = 6$), the larger the side-chain, the higher the Po. In contrast, for hydrophobic ones such as alanine ($0.60 \pm 0.02$, $n = 6$), isoleucine ($0.19 \pm 0.03$, $n = 6$) and valine ($0.44 \pm 0.05$, $n = 7$), the larger the side-chain, the lower the Po. Single-channel kinetic analysis revealed that mutations mainly affect the open time. To exclude possible effects of the mutation on ATP hydrolysis, we introduced some mutations into the E1371S background, whose ATP hydrolysis is abolished. Interestingly, mutations that shorten the open time under the wild-type background also decrease significantly the Po in E1371S. The implications of our data on the gating and permeation mechanisms for CFTR will be discussed.

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**PP1 Anchoring onto NCX1 Facilitates Dephosphorylation of P-SER68-PLM**

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**Introduction:** The cardiac $Na^+/Ca^{2+}$ exchanger 1 (NCX1) modulates excitation-contraction coupling and contributes to $Ca^{2+}$ removal in cardiomyocytes. Altered expression and activity of NCX1 is linked to dysfunctional $Ca^{2+}$ handling in chronic heart disease. Consequently, modulation of NCX1 activity is proposed as a therapeutic target. The cytosolic loop of NCX1, ~550 amino acids, comprises several important docking and regulation sites. In particular, phospholemman (PLM) has been shown to interact and inhibit NCX1 activity when phosphorylated at serine 68 (pSer68-PLM). Importantly, pSer68-PLM has been shown as a substrate for protein phosphatase 1 (PP1) in the context of PLM regulation of the sodium-potassium pump. PP1 regulation of NCX1 is unknown.

**Hypothesis:** PP1 binds to NCX1 and regulates its activity by dephosphorylating pSer68-PLM.

**Methods and Results:** Using co-immunoprecipitation in rat heart lysates we have shown that NCX1 exists in a macromolecular complex with PP1 and PLM. This facilitates specific control of NCX function. Bioinformatic analysis revealed three putative PP1 binding sites on NCX1. Co-localization studies, co-immunoprecipitations, pull down, mutation- and peptide overlay assays indicated that PP1 bound directly to the consensus sequence R/KVxF in calcium binding domain 1 (CBD1) of NCX1. The reciprocal NCX1 binding site in PP1 was identified within residues 235-260, a region which harbours important anchoring sites. A peptide docking model was generated showing how the R/KVxF peptide may bind in the hydrophobic pocket of PP1. Surface plasmon resonance analysis indicated that the NCX1-PP1 binding is strong and stable. This binding does not inhibit PP1 activity. Co-expression of NCX1 with PLM and PP1 in HEK293 down regulates pSer68-PLM, indicating that PP1-R/KVxF- binding is a prerequisite for dephosphorylation of pSer68-PLM.

**Conclusion:** R/KVxF motif in NCX1-CBD1 anchors PP1, does not change the activity of the enzyme and facilitates dephosphorylation of pSer68-PLM.

**2951-Pos Board B381**

**The Pore-Domain of TRPA1 Mediates the Inhibitory Effect of the Antagonist 6-Methyl-5-(2-(Trifluoromethyl)Phenyl)-1H-Indazole**

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The pore-domain of TRPA1 mediates the inhibitory effect of the antagonist 6-Methyl-5-(2-(trifluoromethyl)phenyl)-1H-indazole

The ion channel TRPA1 gives to the organisms the ability to detect noxious chemicals to sensory neurons and as a result of this, mediates chemical nociception in vivo. Mouse TRPA1 is activated by electrophilic compounds such as mustard-oil (MO) and several physical stimuli such as cold temperature. Both stimuli are mechanistic linked to the N terminus of the channel. Due to its sensory function, the inhibition of TRPA1 activity might provide an effective treatment against chronic and inflammatory pain, for that reason, TRPA1 has become an important target for the development of new and better analgesic drugs. 6-Methyl-5-(2-(trifluoromethyl)phenyl)-1H-indazole (Compound 31) has been identified by a chemical screen and lead optimization as an inhibitor of chemical activation of TRPA1. However, the structures or domains of TRPA1 that mediate the inhibitory effect of Compound 31 are unknown. Here, we screened 12,000 random mutant clones of mouse TRPA1 for their sensitivity to mustard-oil and the ability of Compound 31 to inhibit chemical activation by MO. We identified five mutations located within the pore domain that cause loss of inhibition by Compound 31, one of them in the residue T877 placed in the TM5, important in the binding of menthol, a dose-dependent agonist/blocker. This result demonstrates that the pore-domain is a regulator of chemical activation and suggests that Compound 31 might be acting directly on the pore-domain.

**2952-Pos Board B382**

**Planar Patch Clamp System Capable of Recording Mechanosensitive Activity of Ion Channels**

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Response to mechanical stimuli, which includes proper development of biological tissues and organs, is essential to the cell. Mechanosensitive ion channels are the basic elements among mechanosensing molecules and complexes. Although the traditional patch clamp technique using glass micropipettes is still widely used for electrophysiology, it is difficult to control the mechanical stimulus to ion channels in this system. Here we developed a novel patch clamp system with a planar stretchable electrode to record mechanosensitive responses of ion channels. Planar electrodes of 100 μm thickness were fabricated using silicone resin. Next, we prepared HEK293 cells transfected with the stretch-activated KCa (SAKCA) channel, which is mechanosensitive, for planar patch clamp recordings. Using our planar patch clamp system, a gigaohm seal was achieved with a maximum seal resistance of 10 GΩ. The success rate of gigaohm seal formation was 37%. Then we recorded single channel currents with a slope conductance of 285.4 pS, which is parallel to the known SAKCA current. Using scanning electron microscope, we confirmed elongation of the aperture by 37.7% when 50% stretch was applied to the planar electrode. It is expected that a controllable and sufficient stretch stimulus can be applied to the cellular membrane using our newly developed planar patch clamp system.

**2953-Pos Board B383**

**Single-Channel Analysis of the Molecular Pharmacology of the Long QT Syndrome Variant 3**

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The Long QT Syndrome (LQTS) is characterized by a prolongation of the QT interval on an ECG and occurrences of ventricular fibrillation, polymorphic ventricular tachycardia, and sudden cardiac death. In patients with LQTS variant 3 (LQT3), mutations in the cardiac sodium channel alpha subunit, Nav1.5, disrupt channel inactivation by multiple mechanisms and can cause a sustained depolarizing current ($I_{NaL}$) sufficient to prolong the ventricular action potential. LQT3 mutant sodium channels are therefore a reliable experimental model for the study of the function and pharmacology of dysfunctional